TOTAL RNA ISOLATION FROM CELL LINE PROTOCOL

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Total RNA Isolation From Cell Line Protocol

1. Thaw cell lysate in a water bath at room temperature (25°C).
2. Add 0.2 ml chloroform per 1ml lysate.
3. Shake each tube vigorously for 15 seconds & store at room temperature for 15 minutes.
4. Centrifuge for 20 minutes at 12,000 g.
5. Transfer the aqueous phase to a fresh tube and add 10ul of Glycogen. Add 0.5ml of isopropanol per 1ml cell lysate. Mix very well.
6. Store at room temperature for 10 min. Centrifuge for 30 min. at 12,000 g.
7. Remove supernatant. Air dry briefly. Add 1ml of 75% ethanol per 1ml lysate. Spin for 30 min. at 12,000 g.
8. Rehydrate in DEPC treated water (amount depends on the size of the RNA pellet)
9. Heat at 65C for 5 minutes and then mix very well. Centrifuge for 5 seconds.
10. Read OD in 10mM Tris HCl pH 7.5 after 1:100 dilution
11. Determine the RNA concentration using the results from the OD reading and the total volume of RNA.
12. Take out 5 ul for RNA chip analysis and store the remaining RNA at -70°C
13. Re-precipitate RNA by adding 1/10 volume of 3 M Sodium Acetate and 2.5-volume alcohol and store at -20°C.

EXAMPLE OF DATA ACQUISITION AND ANALYSIS